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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/699,848	11/04/2003	Tetsuo Yukimasa	61352-0052	7282

7590 10/24/2006

Michael E. Fogarty
McDERMOTT, WILL & EMERY
600 13th Street, N.W.
Washington, DC 20005-3096

EXAMINER	
PANDE, SUCHIRA	
ART UNIT	**PAPER NUMBER**
1637	

DATE MAILED: 10/24/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/699,848	YUKIMASA ET AL.	
	Examiner	Art Unit	
	Suchira Pande	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on _____.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2 and 4-10* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-2 and 4-10* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date *6/20/2006*.
4) ☒ Interview Summary (PTO-413) Paper No(s)/Mail Date. *10182006*.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The Office action mailed on June 20, 2006 is vacated.

Amendment

1. This Office action is in response to Amedment to claims filed on June 20, 2006. Claims 1, 5,7 and 9 have been amended. Claim 3 has been cancelled. Currently claims 1-2, 4-10 are pending in this application and are being examined.

Priority

2. The applicant has not provided English translation of foreign Japanese Application 2002-077359 filed on March 19, 2002. Hence for the purposes of examination of the current application, the claims are considered to be entitled to the priority date of filing of the PCT application, March 17, 2003.

Claim Rejections - 35 USC § 103

3. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

4. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was

not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

5. Claims 1-2 and 4 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scopes (1972) Analytical Biochemistry 49, 88-94 in view of Imamura, S. and Ikura, Y. (2000) JP Pat. 2000-189188 (cited in OA of June 20, 2006) and Alvarez-Gonzalez et. al. (2000) Anal. Chem. Vol. 72, pp. 520-527 as evidenced by Carlier, M-F (1987) Biochem. and Biophys. Res. Comm. Vol. 143 (3) pages 1069-1075 (cited in IDS).

A) Regarding claim 1, Scopes describes:

a. A method for detecting an inorganic phosphoric acid (see page 88, par. 2)

b. Sample including;

i. glyceraldehyde-3-phosphate (See chemical expression 1 in page 88, par. 2-3).

ii. oxidized nicotinamide adenine dinucleotide (NAD^+). See page 88, chemical expression 1. The oxidized NAD^+ acts as coenzyme in the forward reaction where glyceraldehyde-3-phosphate gets converted into 1,3-diphosphoglycerate concomitant with the reduction of coenzyme to NADH.

iii. glyceraldehyde phosphate dehydrogenase. See page 88 chemical expression 1 where this enzyme mediates the conversion of

glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate in presence of Pi and NAD^+.

Regarding claim 4, Scopes teaches a method of detecting inorganic phosphoric acid that comprises adenosine diphosphate and phosphoglycerate kinase. See page 88, par. 3 and chemical expression 2 where 1,3 –diphosphoglycerate combines with ADP in presence of enzyme phosphoglycerate kinase to form 3-phosphoglycerate and ATP.

It should be noted that in the reaction taught by Scopes the concentration of Pi is actually measured by detection of NADH produced (see Page 89, Fig. I and lines 1-2 of par. 2). For each molecule of Pi used in the reaction one molecule of NADH is generated as shown in page 89 and the concentration of NADH is measured at 340 nm using spectrophotometer. The system of measurement of NADH as taught by Scopes is capable of measuring Pi down to as low as 1μM (see page 93 par. 3).

B) Regarding claim 1, Scopes does not teach:

c. diaphorase

d. an electron mediator

e. measuring a current value in said measurement system. They measure the concentration of inorganic phosphoric acid by reading absorbance using a spectrophotometer. See page 88 par. 4.

C) Regarding claim 1, Imamura and Ikura (2000) JP Pat. 2000-189188 (English Translation Provided), teach an analysis method where following components are described:

f. oxidized nicotinamide adenine dinucleotide phosphate NAD^+. They describe an enzyme cycling signal amplifying reaction system requiring NAD^+ and its reduction to NADH. See page 2, claim 2.

g. an electron mediator. See page 11, par. 0011 where aminophenols, ferrocene, and benzoquinones are taught as electron mediators. Also see page 17, par. 0019.

h. and measuring a current value using the measurement system, wherein said current value indicates the concentration of the inorganic phosphoric acid in said sample. See page 22 par. 0028, where Imamura and Ikura describe a method to analyze the conversion of NAD^+ to NADH, which is measured as electrical current in nano Amperes (nA). The reduced NADH is converted back into oxidized NAD+ by the electron mediator. See pages 5 and 6 par. 0003. The system can quantitatively analyze conversion of NAD^+ to NADH with very high sensitivity in terms of the current value expressed in nA. (See page 8, par. 0008; page 9, par. 0009-0010 and page 22, fig. 1)

D) Regarding claim 1, Imamura and Ikura do not teach the substrate and enzyme combination of: glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase. But they teach that their invention pertains to enzymatic analysis method useful for diverse processes where NAD or NADH acts as cofactor (See page 6, par. 0004 and page 7, par. 0006). In addition they describe the shortcomings in terms of low sensitivity of the existing methods of measuring NAD or NADH using

spectroscopic measurements where enzyme reactions had to be carried out in an aqueous solution and high –precision analyzer was essential (see page 8, par 0006).

Regarding claim 1, Imamura and Ikura teach a measurement system comprising diaphorase. See page 2, claims 1-3 where diaphorase, a compound having an electron transport function is claimed.

Regarding claim 2, Imamura and Ikura teach electron mediators selected from a group comprised of aminophenols, ferrocene, and benzoquinones (see page 10, par. 0011). They teach use of quinones such as 1,4-napthoquinone, phenols such as 2,6-dichlorophenolindopenol, ferrocenes such iron cyanides of potassium ferrocyanide (see page 17, par. 0019).

Method of Scopes can measure the inorganic phosphate concentration with good precision but requires the use of bulky spectrophotometer (page 88 par. 4) and 10^{-9} mole of Pi in a sample could be detected (See Table 1 on page 91 showing detection of 30-100 μM Pi). Combining it with the method of Imamura and Ikura allow them to add the additional advantage of not being dependent on use of a bulky analyzer to determine the concentration of inorganic phosphoric acid. Instead of measuring amount of NADH generated by measuring absorbance at 340nm and determining the concentration of Pi using formula shown in Table 1 page 91 now the measurement of generated NADH is done rapidly, with precision using highly sensitive electrical signal generated as a result of formation of NADH. For each molecule of inorganic phosphorous that reacts with glyceraldehyde-3-phosphate a molecule of NAD+ is

converted to NADH. By measuring concentration of NADH directly concentration of Pi is determined using this electrical measurement.

Imamura, S. and Ikura, Y provide the motivation to combine the two methods they state "The purpose of the present invention is to provide an enzyme cycling reagent for simply and rapidly detecting NAD or NADH concentration with high sensitivity and high precision without using a complicated analyzer" (See page 8, par. 0007).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to use the method of electrical NADH based detection taught by Imamura, S. and Ikura, Y in the method of NADH based inorganic phosphate detection taught by Scopes.

E) Regarding claim 1, neither Scopes nor Imamura, S. and Ikura, Y teach: The inorganic phosphoric acid is detected within 100 seconds after subjecting said sample to the measurement system.

Regarding claim 1, Alvarez-Gonzalez et. al. teach: Detection of NADH within 100 seconds after subjecting said sample to the measurement system (see page 527 figure 7, first par. where using an electrocatalytic method of detecting NADH amperometrically is taught). They teach the response time for 95% of the steady-state current was 75 s. ie NADH formed as a result of the addition of substrate is detected within 100 seconds after subjecting said sample to the measurement system.

Alvarez-Gonzalez et. al. use glycerol and appropriate dehydrogenase in their measurement system to show that current is detected within 100 seconds after

subjecting said sample to the measurement system. They do not use glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase. Carlier provides the evidence that glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase system is even more efficient because the presence of Pi in this NADH based fluorimetric system is detected within 10 sec (see Carlier, M-F page 1071, par. 1 and Figure 1).

It would have been obvious to one of ordinary skill in the art to at the time of the invention to practice the method taught by Alvarez-Gonzalez for glycerol and appropriate dehydrogenase pair in the method of Scopes & Imamura, S. and Ikura, Y. The motivation to do so is provided by Gorton and Dominguez (2002) Reviews in Molecular Biotechnology vol. 82 pp, 371-392. Gorton and Dominguez while describing amperometric biosensors based on NAD(P)-dependent dehydrogenase enzymes on page 386, par. 2 state "To be able to construct a biosensor measuring the production of NADH, it is therefore essential that the NADH formed is instantaneously consumed by the mediator (or possibly directly at the electrode surface), otherwise the equilibrium of Eq. (23) will be reached and further production of NADH will cease. The reduced mediator in turn must also be rapidly reoxidized to recreate its active oxidized form. In essence, this means that all three reaction steps (enzymatic, mediated and electrochemical) need to occur very close in space for a successful approach.-------It is therefore very important that the mediated reaction is as rapid as possible, and this motivates the ongoing search for an optimal mediator."

6. Claims 5 and 6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Scopes (1972) Analytical Biochemistry 49, 88-94; Imamura, S. and Ikura, Y. (2000) JP Pat. 2000-189188 (cited in OA of June 20, 2006) & Alvarez-Gonzalez et. al. (2000) Anal. Chem. Vol. 72, pp. 520-527 as evidenced by Carlier, M-F (1987) Biochem. and Biophys. Res. Comm. Vol. 143 (3) pages 1069-1075 (cited in IDS) and Baykov and Avaeva (1982) Analytical Biochemistry 119: pp211-213 as evidenced by Baykov and Avaeva (1981) Analytical Biochemistry 116: pp 1-4 (cited in OA of June 20, 2006).

A) Regarding claim 5, Scopes describes:

i. A method for detecting an inorganic phosphoric acid (see page 88, par. 2)

j. Sample including;

iv. glyceraldehyde-3-phosphate (See chemical expression 1 in page 88, par. 2-3).

v. oxidized nicotinamide adenine dinucleotide (NAD^+). See page 88, chemical expression 1. The oxidized NAD^+ acts as coenzyme in the forward reaction where glyceraldehyde-3-phosphate gets converted into 1,3-diphosphoglycerate concomitant with the reduction of coenzyme to NADH.

vi. glyceraldehyde phosphate dehydrogenase. See page 88 chemical expression 1 where this enzyme mediates the conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate in presence of Pi and NAD^+.

It should be noted that in the reaction taught by Scopes the concentration of Pi is actually measured by detection of NADH produced (see Page 89, Fig. 1 and lines 1-2 of par. 2). For each molecule of Pi used in the reaction one molecule of NADH is generated as shown in page 89 and the concentration of NADH is measured at 340 nm using spectrophotometer. The system of measurement of NADH as taught by Scopes is capable of measuring Pi down to as low as 1μM (see page 93 par. 3).

B) Regarding claim 5, Scopes does not teach:

k. diaphorase

l. an electron mediator

m. measuring a current value in said measurement system, wherein said current value indicates the concentration of the pyrophosphate in said sample. They measure the concentration of inorganic phosphoric acid by reading absorbance using a spectrophotometer. See page 88 par. 4.

C) Regarding claim 5, Imamura and Ikura (2000) JP Pat. 2000-189188 (English Translation Provided), teach an analysis method where following components are described:

n. oxidized nicotinamide adenine dinucleotide phosphate NAD^+. They describe an enzyme cycling signal amplifying reaction system requiring NAD^+ and its reduction to NADH. See page 2, claim 2.

o. diaphorase. See page 2, claims 1-3 where diaphorase, a compound having an electron transport function is claimed.

p. an electron mediator. See page 11, par. 0011 where aminophenols, ferrocene, and benzoquinones are taught as electron mediators. Also see page 17, par. 0019.

q. and measuring a current value using the measurement system, wherein said current value indicates the concentration of the inorganic phosphoric acid in said sample. See page 22 par. 0028, where Imamura and Ikura describe a method to analyze the conversion of NAD^+ to NADH, which is measured as electrical current in nano Amperes (nA). The reduced NADH is converted back into oxidized NAD+ by the electron mediator. See pages 5 and 6 par. 0003. The system can quantitatively analyze conversion of NAD^+ to NADH with very high sensitivity in terms of the current value expressed in nA. (See page 8, par. 0008; page 9, par. 0009-0010 and page 22, fig. 1)

D) Regarding claim 5, Imamura and Ikura do not teach the substrate and enzyme combination of: glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase. But they teach that their invention pertains to enzymatic analysis method useful for diverse processes where NAD or NADH acts as cofactor (See page 6, par. 0004 and page 7, par. 0006). In addition they describe the shortcomings in terms of low sensitivity of the existing methods of measuring NAD or NADH using spectroscopic measurements where enzyme reactions had to be carried out in an aqueous solution and high –precision analyzer was essential (see page 8, par 0006).

Method of Scopes can measure the inorganic phosphate concentration with good precision but requires the use of bulky spectrophotometer (page 88 par. 4) and 10^{-9}

mole of Pi in a sample could be detected (See Table 1 on page 91 showing detection of 30-100 μM Pi). Combining it with the method of Imamura and Ikura allow them to add the additional advantage of not being dependent on use of a bulky analyzer to determine the concentration of inorganic phosphoric acid. Instead of measuring amount of NADH generated by measuring absorbance at 340nm and determining the concentration of Pi using formula shown in Table 1 page 91 now the measurement of generated NADH is done rapidly, with precision using highly sensitive electrical signal generated as a result of formation of NADH. For each molecule of inorganic phosphorous that reacts with glyceraldehyde-3-phosphate a molecule of NAD+ is converted to NADH. By measuring concentration of NADH directly concentration of Pi is determined using this electrical measurement.

Imamura, S. and Ikura, Y provide the motivation to combine the two methods they state "The purpose of the present invention is to provide an enzyme cycling reagent for simply and rapidly detecting NAD or NADH concentration with high sensitivity and high precision without using a complicated analyzer" (See page 8, par. 0007).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to use the method of electrical NADH based detection taught by Imamura, S. and Ikura, Y in the method of NADH based inorganic phosphate detection taught by Scopes.

E) Regarding claim 5, neither Scopes nor Imamura, S. and Ikura, Y teaches: detection within 100 seconds after subjecting said sample to the measurement system.

Regarding claim 5, Alvarez-Gonzalez et. al. teach: Detection of NADH within 100 seconds after subjecting said sample to the measurement system (see page 527 figure 7, first par. where using an electrocatalytic method of detecting NADH amperometrically is taught). They teach the response time for 95% of the steady-state current was 75 s. ie NADH formed as a result of the addition of substrate is detected within 100 seconds after subjecting said sample to the measurement system.

Alvarez-Gonzalez et. al. use glycerol and appropriate dehydrogenase in their measurement system to show that current is detected within 100 seconds after subjecting said sample to the measurement system. They do not use glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase. Carlier provides the evidence that glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase system is even more efficient because the presence of Pi in this NADH based fluorimetric system is detected within 10 sec (see Carlier, M-F page 1071, par. 1 and Figure 1).

It would have been obvious to one of ordinary skill in the art to at the time of the invention to practice the method taught by Alvarez-Gonzalez for glycerol and appropriate dehydrogenase pair in the method of Scopes & Imamura, S. and Ikura, Y. The motivation to do so is provided by Gorton and Dominguez (2002) Reviews in Molecular Biotechnology vol. 82 pp, 371-392. Gorton and Dominguez while describing amperometric biosensors based on NAD(P)-dependent dehydrogenase enzymes on page 386, par. 2 state "To be able to construct a biosensor measuring the production of NADH, it is therefore essential that the NADH formed is instantaneously consumed by

the mediator (or possibly directly at the electrode surface), otherwise the equilibrium of Eq. (23) will be reached and further production of NADH will cease. The reduced mediator in turn must also be rapidly reoxidized to recreate its active oxidized form. In essence, this means that all three reaction steps (enzymatic, mediated and electrochemical) need to occur very close in space for a successful approach.-------It is therefore very important that the mediated reaction is as rapid as possible, and this motivates the ongoing search for an optimal mediator."

E) Regarding claim 5, Scopes; Imamura and Ikura; & Alvarez-Gonzalez do not teach:

r. a method of detecting a pyrophosphate which comprises:

s. converting the pyrophosphate in a sample into an inorganic acid.

F) Regarding claim 5, Baykov and Avaeva (1982) teaches a simple method for measuring pyrophosphate (PPi) (see Baykov and Avaeva (1982) page 211, par. 4-7; page 212 par. 2 and Table 1). The conversion of pyrophosphate into inorganic acid is further evidenced in Baykov and Avaeva (1981) where they describe conversion of pyrophosphate into phosphoric acid (see Baykov and Avaeva (1981) page 1, par. 2; page 2, par. 2 & legend of Fig. 2; and page 3, Fig. 3).

Regarding claim 6, Baykov and Avaeva (1982) teach use of inorganic pyrophosphatase in the method of measuring PPi (see Baykov and Avaeva (1982) page 211 abstract). This is further evidenced in Baykov and Avaeva (1981) where use of pyrophosphatase to convert pyrophosphate into inorganic phosphoric acid is explicitly taught (see page 1, par. 2; and page 3 Fig. 4).

It would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to use method of detecting pyrophosphate by converting pyrophosphate into phosphoric acid taught by Baykov and Avaeva in the method of Scopes and Imamura & Ikura. The motivation to do so is provided by Baykov and Avaeva (1982) who state " A simple method of measuring PPi at concentrations down to 2 μM has been devised -----. Orthophosphate (20mM) and a number of other compounds did not interfere with the assay. The applicability of the method for direct measurement of PPi in urine is demonstrated " (see page 211 abstract). They further point out the reasons why the analysis of PPi in the presence of Pi is a problem in biochemistry and clinical chemistry (page 211 par. 1) and how their method of measuring PPi by converting it to Pi and measuring the Pi produced can give accurate measurement even in presence of interfering compounds. (See page 212 last par.; page 213 par. 1 and 2). The combination of their method would allow the applicants to accurately measure the PPi concentrations in samples of interest to them even in presence of interfering compounds.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to use method of detecting pyrophosphate by converting pyrophosphate into phosphoric acid taught by Baykov and Avaeva in the method of Scopes and Imamura & Ikura.

7. Claims 7-10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nyren (2001) US Pat. 6,258,568; Baykov and Avaeva (1981) Analytical Biochemistry

116: pp 1-4 as evidenced by Baykov and Avaeva (1982) Analytical Biochemistry 119: pp211-213; Scopes (1972) Analytical Biochemistry 49, 88-94; Imamura, S. & Ikura, Y. (2000) JP Pat. 2000-189188 (cited in OA of June 20, 2006); and Alvarez-Gonzalez et. al. (2000) Anal. Chem. Vol. 72, pp. 520-527 as evidenced by Carlier, M-F (1987) Biochem. and Biophys. Res. Comm. Vol. 143 (3) pages 1069-1075 (cited in IDS).

A) Regarding claims 7-10 Nyren teaches:

t. A method of detecting a nucleic acid which comprises:

u. subjecting a sample to a reaction system including a DNA probe having a complementary sequence to the sequence of the said nucleic acid, DNA polymerase and a deoxynucleotide and allowing extension of said DNA probe whereby a pyrophosphate produced concurrent with the extension reaction of said DNA probe. (see col.17, lines 35-50 and col. 18, lines 22-33).

Regarding claims 8 and 10 Nyren teaches extension reaction of the DNA probe by PCR reaction (see col. 8 lines 9-30).

Regarding claim 9 Nyren teaches a method of typing a SNP sequence of a DNA (see col. 10, lines 49-62).

B) Regarding claims 7-10 Nyren does not teach:

v. converting the pyrophosphate produced in the sample into an inorganic phosphoric acid;

w. subjecting said sample to a measurement system including glyceraldehyde-3-phosphate, oxidized nicotinamide adenine dinucleotide or

oxidized nicotinamide adenine dinucleotide phosphate, glyceraldehyde phosphate dehydrogenase, diaphorase and an electron mediator; and

x. measuring a current value in said measurement system, wherein said current value indicates the concentration of the nucleic acid having a specified sequence in said sample, and the nucleic acid is detected within 100 seconds after subjecting said sample to the measurement system.

C) Regarding claims 7-10 Baykov and Avaeva (1981) teaches a method for converting pyrophosphate (PPi) produced in the sample into inorganic phosphoric acid (see Baykov and Avaeva (1981) page 1, par. 2; page 2, par. 2 & legend of Fig. 2; and page 3, Fig. 3).

Nyren points out a problem that is associated with PPi-based sequencing methods where dATP (one of the 4 dNTPs) used in the chain extension DNA sequencing reaction, interferes with the subsequent luciferase-based detection of PPi (see col. 7, lines 14-23). A sensitive method of PPi detection where measurement can be made reliably and accurately in presence of dATP or other compounds and ions that are present in samples to be analyzed would provide a solution for the above identified problem.

Baykov and Avaeva (1981) as evidenced by Baykov and Avaeva (1982) provide the perfect solution to the problem identified by Nyren. Baykov and Avaeva (1981) state "Despite the obvious advantage which a continuous assay has over a fixed –time assay for kinetic studies of chemical processes, the former is rarely used for the reactions resulting in phosphate production or consumption. This is explained by the absence of

inexpensive analyzers of Pi suitable for routine kinetic work." (see page 1, par. 1). They further teach the advantages associated with their method of converting PPi into Pi and its measurement by the apparatus used by them. This method is capable of measuring relatively fast reactions (see Baykov and Avaeva, page 3, par. 2) without deviation from linearity and is capable of measuring Pi in presence of other compounds such as ATP, ADP, etc. (see Baykov and Avaeva, page 3, par. 3). The combination of their method where PPi is converted into Pi using pyrophosphatase and resulting Pi concentration measured would allow the applicants to accurately measure the PPi concentrations in samples of interest to them even in presence of acid labile compounds such as the dNTPs without any interference. This indeed is the case as is corroborated and further evidenced by Baykov and Avaeva (1982) who state " A simple method of measuring PPi at concentrations down to 2 μM has been devised -----. Orthophosphate (20mM) and a number of other compounds did not interfere with the assay. The applicability of the method for direct measurement of PPi in urine is demonstrated " (see page 211 abstract). They further point out the reasons why the analysis of PPi in the presence of Pi is a problem in biochemistry and clinical chemistry (page 211 par. 1) and how their method of measuring PPi by converting it to Pi and measuring the Pi produced can give accurate measurement even in presence of interfering compounds. (See page 212 last par.; page 213 par. 1 and 2). The combination of their method would allow the applicants to accurately measure the PPi concentrations in samples of interest to them even in presence of interfering compounds.

Therefore it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to use method of converting pyrophosphate into phosphoric acid taught by Baykov and Avaeva in the method of Nyren.

D) Regarding claims 7-10 Nyren and Baykov & Avaeva do not teach:

y. subjecting said sample to a measurement system including glyceraldehyde-3-phosphate, oxidized nicotinamide adenine dinucleotide or oxidized nicotinamide adenine dinucleotide phosphate, glyceraldehyde phosphate dehydrogenase,

z. diaphorase

aa. and an electron mediator;

bb. and measuring a current value in said measurement system, wherein said current value indicates the concentration of the nucleic acid having a specified sequence in said sample, and the nucleic acid is detected within 100 seconds after subjecting said sample to the measurement system.

E) Regarding claims 7-10 Scopes describes:

cc. A method for detecting an inorganic phosphoric acid (see page 88, par. 2)

dd. Subjecting said sample to a measurement system including;

vii. glyceraldehyde-3-phosphate (See chemical expression 1 in page 88, par. 2-3).

viii. oxidized nicotinamide adenine dinucleotide (NAD^+). See page 88, chemical expression 1. The oxidized NAD^+ acts as coenzyme in the

forward reaction where glyceraldehyde-3-phosphate gets converted into 1,3-diphosphoglycerate concomitant with the reduction of coenzyme to NADH.

ix. glyceraldehyde phosphate dehydrogenase. See page 88 chemical expression 1 where this enzyme mediates the conversion of glyceraldehyde-3-phosphate to 1,3-diphosphoglycerate in presence of Pi and NAD^+.

It should be noted that in the reaction taught by Scopes the concentration of Pi is actually measured by detection of NADH produced (see Page 89, Fig. I and lines 1-2 of par. 2). For each molecule of Pi used in the reaction one molecule of NADH is generated as shown in page 89 and the concentration of NADH is measured at 340 nm using spectrophotometer. The system of measurement of NADH as taught by Scopes is capable of measuring Pi down to as low as 1μM (see page 93 par. 3).

F) Regarding claim 7-10, Scopes does not teach:

ee. diaphorase

ff. an electron mediator

gg. measuring a current value in said measurement system. They measure the concentration of inorganic phosphoric acid by reading absorbance using a spectrophotometer. See page 88 par. 4.

G) Regarding claims 7-10 , Imamura and Ikura (2000) JP Pat. 2000-189188 (English Translation Provided), teach an analysis method where following components are described:

hh. oxidized nicotinamide adenine dinucleotide phosphate NAD^+. They describe an enzyme cycling signal amplifying reaction system requiring NAD^+ and its reduction to NADH. See page 2, claim 2.

ii. diaphorase. See page 2, claims 1-3 where diaphorase, a compound having an electron transport function is claimed.

jj. an electron mediator. See page 11, par. 0011 where aminophenols, ferrocene, and benzoquinones are taught as electron mediators. Also see page 17, par. 0019.

kk. and measuring a current value using the measurement system, wherein said current value indicates the concentration of the inorganic phosphoric acid in said sample. See page 22 par. 0028, where Imamura and Ikura describe a method to analyze the conversion of NAD^+ to NADH, which is measured as electrical current in nano Amperes (nA). The reduced NADH is converted back into oxidized NAD+ by the electron mediator. See pages 5 and 6 par. 0003. The system can quantitatively analyze conversion of NAD^+ to NADH with very high sensitivity in terms of the current value expressed in nA. (See page 8, par. 0008; page 9, par. 0009-0010 and page 22, fig. 1)

G) Regarding claims 7-10, Imamura and Ikura do not teach the substrate and enzyme combination of: glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase. But they teach that their invention pertains to enzymatic analysis method useful for diverse processes where NAD or NADH acts as cofactor (See page 6, par. 0004 and page 7, par. 0006). In addition they describe the shortcomings in terms

of low sensitivity of the existing methods of measuring NAD or NADH using spectroscopic measurements where enzyme reactions had to be carried out in an aqueous solution and high –precision analyzer was essential (see page 8, par 0006).

Method of Scopes can measure the inorganic phosphate concentration with good precision but requires the use of bulky spectrophotometer (page 88 par. 4) and 10^{-9} mole of Pi in a sample could be detected (See Table 1 on page 91 showing detection of 30-100 μM Pi). Combining it with the method of Imamura and Ikura allow them to add the additional advantage of not being dependent on use of a bulky analyzer to determine the concentration of inorganic phosphoric acid. Instead of measuring amount of NADH generated by measuring absorbance at 340nm and determining the concentration of Pi using formula shown in Table 1 page 91 now the measurement of generated NADH is done rapidly, with precision using highly sensitive electrical signal generated as a result of formation of NADH. For each molecule of inorganic phosphorous that reacts with glyceraldehyde-3-phosphate a molecule of NAD+ is converted to NADH. By measuring concentration of NADH directly concentration of Pi is determined using this electrical measurement.

Imamura, S. and Ikura, Y provide the motivation to combine the methods they state "The purpose of the present invention is to provide an enzyme cycling reagent for simply and rapidly detecting NAD or NADH concentration with high sensitivity and high precision without using a complicated analyzer" (See page 8, par. 0007).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the claimed invention was made to use the method of electrical NADH

based detection taught by Imamura, S. and Ikura, Y in the method of NADH based inorganic phosphate detection taught by Scopes to detect the nucleic acid in the method of Nyren and Baykov & Avaeva. The advantage of this combination would be that extension of nucleic acids such as DNA chains, SNP polymorphisms, and PCR reactions which all are accompanied by release of PPi every time a dNTP is added to the growing DNA chain could be detected rapidly without requiring the use of any kind of spectrophotometric / fluorescent analyzers.

E) Regarding claims 7-10, neither Nyren and Baykov & Avaeva nor Scopes & Imamura, S. and Ikura, Y teach: detection within 100 seconds after subjecting said sample to the measurement system.

Regarding claims 7-10, Alvarez-Gonzalez et. al. teach: Detection of NADH within 100 seconds after subjecting said sample to the measurement system (see page 527 figure 7, first par. where using an electrocatalytic method of detecting NADH amperometrically is taught). They teach the response time for 95% of the steady-state current was 75 s. ie NADH formed as a result of the addition of substrate is detected within 100 seconds after subjecting said sample to the measurement system.

Alvarez-Gonzalez et. al. use glycerol and appropriate dehydrogenase in their measurement system to show that current is detected within 100 seconds after subjecting said sample to the measurement system. They do not use glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase. Carlier provides the evidence that glyceraldehyde-3-phosphate and glyceraldehyde phosphate dehydrogenase system is even more efficient because the presence of Pi in this NADH

based fluorimetric system is detected within 10 sec (see Carlier, M-F page 1071, par. 1 and Figure 1).

It would have been obvious to one of ordinary skill in the art to at the time of the invention to practice the method taught by Alvarez-Gonzalez for glycerol and appropriate dehydrogenase pair in the method of Nyren; Baykov & Avaeva; Scopes; and Imamura, S. & Ikura, Y. The motivation to do so is provided by Gorton and Dominguez (2002) Reviews in Molecular Biotechnology vol. 82 pp, 371-392. Gorton and Dominguez while describing amperometric biosensors based on NAD(P)-dependent dehydrogenase enzymes on page 386, par. 2 state "To be able to construct a biosensor measuring the production of NADH, it is therefore essential that the NADH formed is instantaneously consumed by the mediator (or possibly directly at the electrode surface), otherwise the equilibrium of Eq. (23) will be reached and further production of NADH will cease. The reduced mediator in turn must also be rapidly reoxidized to recreate its active oxidized form. In essence, this means that all three reaction steps (enzymatic, mediated and electrochemical) need to occur very close in space for a successful approach.-------It is therefore very important that the mediated reaction is as rapid as possible, and this motivates the ongoing search for an optimal mediator."

Conclusion

All claims 1-2, 4-10 are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Suchira Pande whose telephone number is 571-272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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Suchira Pande
Examiner
Art Unit 1637

TERESA E. STRZELECKA, PH.D.
PRIMARY EXAMINER
Teresa Strzelecka
10/19/06